REMARKS

The Official Action dated January 2, 2003 has been carefully considered. Additionally, the telephone interview which the Examiner courteously afforded Applicants' representatives is acknowledged and appreciated. Accordingly, the changes presented herewith, taken with the following remarks, are believed sufficient to place the present application in condition for allowance. Reconsideration is respectfully requested.

Although a formal agreement regarding the claims was not reached during the aforementioned interview, the claim amendments presented herein were discussed and are believed to overcome the outstanding rejections.

By the present amendment, claims 32, 45, 48-49, 54 and 59 are cancelled. Claims 27, 28, 43, 53 and 55 are amended to clarify the limitations therein, specifically to recite that (1) the mammalian cells are "*in vitro*"; (2) the prokaryotic beta recombinase is derived from *Streptococcus*; (3) the method comprises two steps namely, transfecting prokaryotic beta recombinase and integrating/transfecting the DNA sequence containing the *six* sites in the *in vitro* mammalian cells; and (4) recombination occurs between *six* sites, in accordance with the teachings in the specification, including the Examples. Claims 33, 35-40 and 50 are amended as to matters of form, specifically to amend their dependency. Finally, claims 41, 42, 56 and 57 are also amended to clarify the limitations therein. A Version With Markings Showing Changes Made is attached. Claims 66 and 67 are added. Support for these claims may be found throughout the specification, particularly at page 4, lines 1-3. It is believed that these changes do not involve any introduction of new matter whereby entry is believed to be in order and is respectfully requested.

Claims 27-28, 32-33, 35-50, 53-57 and 59-65 were objected to as being drawn to a non-elected invention. The Examiner asserted that even though the claims are drawn to "mediating transgenic recombination", they are not limited to the elected invention of

methods of genetic modification in animals. Specifically, the Examiner asserted that the claims do not differentiate the method from the same step which would be used for the delivery of polynucleotides to a subject which is encompassed by Group IV, methods of gene therapy. Applicants traverse Examiner's position, however, to expedite prosecution of this application, claims 27, 28, 43, 53, 55 and 60 have been amended to recite that the claims are directed to genetic modification in "*in vitro*" mammalian cells. Accordingly, it is believed that this objection has been overcome. Reconsideration is respectfully requested.

Claims 27-28, 32-33, 35-50, 53-57 and 59-65 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled by the specification. The Examiner asserted that the specification is not enabling for the use of target *six* sites as extra-chromosomal DNA sequences; the use of prokayotic beta recombinase other than those obtain from *Streptococcus*; and the use of methods in a transgenic animal *in vivo*.

However, as will be set forth in detail below, Applicants submit that the methods defined by the present claims are fully enabled to one of ordinary skill in the art, in accordance with the requirements of 35 U.S.C. § 112, first paragraph. Accordingly, this rejection is traversed and reconsideration is respectfully requested.

More particularly, according to independent claim 27, the invention is directed towards methods for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in *in vitro* mammalian cells, comprising the steps of transfecting the mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein recombination occurs between two *six* sites.

According to independent claim 28, the invention is directed to methods for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located

between two *six* sites and inversions of DNA sequences located between two *six* sites, in *in vitro* mammalian cells, comprising the steps of transfecting the mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mammalian cells; wherein recombination occurs between two *six* sites.

According to independent claim 43, the invention is directed to methods for catalyzing site-specific resolution of DNA sequences located between *six* sites in an extrachromosomal substrate transfected into an *in vitro* mammalian cell, comprising the step of catalyzing the site-specific resolution with prokaryotic beta recombinase derived from *Streptococcus*; wherein recombination occurs between *six* sites.

According to independent claim 53, the invention is directed to methods for mediating transgenic intramolecular recombination in *in vitro* mammalian cells, comprising the steps of transfecting mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein recombination occurs between *six* sites and in the presence of cell factors comprising HMG1 chromatin-associated protein.

According to independent claim 55, the invention is directed to methods for mediating transgenic intramolecular recombination in chromatin structures of mammalian cells, comprising the steps of transfecting *in vitro* mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mammalian cells; wherein recombination occurs between *six* sites and in the presence of cell factors comprising HMG1 chromatin-associated protein.

According to independent claim 60, the invention is directed to methods of mediating beta recombinase activity comprising the steps of transfecting *in vitro* mammalian cells with

prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein recombination occurs between *six* sites.

According to independent claim 64, the invention is directed to a method for mediating transgenic intromolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two six sites, in mouse cells, comprising the steps of transfecting mouse cells with prokaryotic beta recombinase derived from Streptococcus and transfecting the mouse cells with DNA sequences containing *six* sites that allow recombination activity; wherein recombination occurs between two *six* sites.

According to independent claim 65, the invention is directed to a method for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two six sites, in mouse cells, comprising the steps of transfecting mouse cells with prokaryotic beta recombinase derived from Streptococcus and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mouse cells; wherein recombination occurs between two *six* sites.

In summary, the methods of claims 27, 28, 53, 55 and 64-65 are directed towards mediating transgenic intermolecular recombination. The methods of claim 43 are directed toward catalyzing site specific resolution, i.e., intramolecular recombination, of DNA sequences located between six sites and an extra chromosomal substrate transfected into a mammalian cell. Finally, the methods of claim 60 are directed towards mediating beta recombinase activity, i.e., intramolecular recombination mediated by the beta recombinase protein. These methods expand the site specific recombinase art by using beta recombinase derived from Streptococcus for mediating intramolecular recombinase reactions between *six*

sites in an *in vitro* mammalian cell or in a mouse cell. As noted by the Examiner, the specification and the art of record teach that prokaryotic beta recombinase is obtained from *Streptococcus* and because of the similarities between strains of *Streptococcus*, an artisan could isolate prokaryotic beta recombinase with the same activity from any strain of *Streptococcus*.

A disclosure is enabling if, from the information set forth in the specification, coupled with information known in the art, one of ordinary skill in the art can make and use the invention without undue experimentation, *United States v. Teletronics, Inc.*, 8 USPQ2d 1217, 1224 (Fed. Cir. 1988). Moreover, every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification; rather, reasonable detail must be provided in order to enable members of the public to understand and carry out the invention, *Genetec v. Novo Nordisc, A/S*, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997). As one of ordinary skill in the art will appreciate that when prokaryotic beta recombinase derived from *Streptococcus* is isolated and specific DNA sequences containing *six* sites that allow recombination activity are identified, the disclosed and claimed methods are fully enabled for *in vitro* mammalian cells or for mouse cells. Accordingly, the specification enables the methods of claims 27, 28, 32-33, 35-50, 53-57 and 59-65 as required by 35 U.S.C. §112, first paragraph. It is therefore submitted that the rejection under 35 U.S.C. §112, first paragraph has been overcome. Reconsideration is respectfully requested.

Claims 27-28, 32-33, 35-50, 53-57 and 59-65 were also rejected under 35 U.S.C. §112, second paragraph as being indefinite. With respect to claims 27, 28, 43, 53, 55, 60 and 64-65, the Examiner asserted that the recitation of "wherein the prokaryotic beta recombinase is capable of using host factors provided by the mammalian cells in order to mediate recombinase activity" is unclear and confusing. Applicants traverse the Examiner's position, however, to expedite prosecution of the application, claims 27-28, 43, 53, 55, 60 and 64-65 have been amended, as suggested by the Examiner in the telephone interview, to recite

"wherein recombination occurs between *six* sites." It is therefore submitted that claims 27, 28, 43, 53, 55, 60 and 64-65 are definite and that the rejection has been overcome.

With respect to claims 27, 28, 43, 53, 55 and 64-65, the Examiner asserted that it is unclear how mediating transgenic intramolecular recombination relates to the single method step of delivering a recombinase in target sequences. Applicants traverse the Examiner's position, however, to expedite prosecution of this application, claims 27, 28, 53, 55 and 64-65 have been amended to specifically recite that the method comprises the steps of transfecting prokaryotic beta recombinase and transfecting or integrating the DNA sequences containing the *six* sites into the *in vitro* mammalian cells. It is therefore submitted that claims 27, 28, 53, 55 and 64-65 are definite and that the rejection has been overcome.

With respect to claims 32, 39 and 40, the Examiner asserted that the claims are unclear by the recitation of the language that a recombination event "is obtained" and further, the Examiner asserted that these claims do not appear to further limit claim 27. Applicants have cancelled claim 32. Moreover, Applicants note that claims 39 and 40 recite specific limitations for claim 27 namely, an intramolecular deletion of a DNA sequence located between direct repeated DNA sequences containing *six* sites (claim 39) and an intramolecular inversion of a DNA sequence located between inverted repeated DNA sequences containing *six* sites (claim 40). Thus claims 39 and 40 further limit claim 27 by reciting specific recombination events which may be obtained from the methods of claim 27. It is therefore submitted that claims 39 and 40 are definite and the rejection has been overcome.

With respect to claim 33, the Examiner asserted that the claim is confusing as to how more than one recombination event is generated in light of the single step recited in claim 27. Applicants note that in claim 27 DNA sequences (plural) containing *six* sites are transfected into the cell and therefore different recombination events may occur with the respective DNA

sequences containing *six* sites. It is therefore submitted that claim 33 is definite and that the rejection has been overcome.

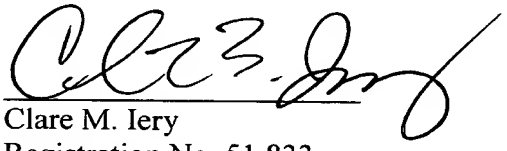
With respect to claims 41, 42 and 44, the Examiner asserted that these claims are confusing because *six* sites are delivered as DNA sequences in claim 27, so it is unclear how stating that they are extrachromosomal further limits claim 27. Applicants traverse the Examiner's position, however, to expedite prosecution, claims 41 and 42 have been amended to recite that "the DNA sequences are located within an extra chromosomal DNA substrate". Moreover, Applicants note that a plasmid, as recited in claim 44, is a specific type of extra-chromosomal substrate and therefore, claim 44 further limits claim 43. It is therefore submitted that claims 41, 42 and 44 are definite, and that the rejection has been overcome.

Finally, the Examiner set forth specific comments with respect to claims 45, 49 and 54. Applicants have cancelled claims 45, 49 and 54.

It is therefore submitted that claims 27-28, 32-33, 35-50, 53-57 and 59-65 are definite and that the rejection under 35 U.S.C. §112, second paragraph has been overcome. Reconsideration is respectfully requested.

It is believed that the above represents a complete response to the objection and rejections under 35 U.S.C. §112, first and second paragraphs, and places the present application in condition for allowance. Reconsideration and an early allowance are requested.

Respectfully submitted,

By: 

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VERSION WITH MARKINGS SHOWING CHANGES MADE

Claims 27, 28, 33, 35-43, 50, 53, 55-57, 60-61 and 64-65 are amended as follows:

27. (Fifth Amendment) A method for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in *in vitro* mammalian cells, comprising the [step] steps of transfecting the mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mammalian cells in order to mediate recombinase activity] recombination occurs between two *six* sites.

28. (Fifth Amendment) A method for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in [chromatin structures of] *in vitro* mammalian cells, comprising the [step] steps of transfecting the mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mammalian cells; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mammalian cells in order to mediate recombinase activity] recombination occurs between *six* sites.

33. (Fourth Amendment) A method according to claim [32] 27, wherein two or more intramolecular recombination events involving different DNA sequences located between different *six* sites occur at the same time.

35. (Twice Amended) A method according to claim [32] 27, wherein an intramolecular deletion of DNA sequences located between directly oriented *six* sites is obtained.

36. (Twice Amended) A method according to claim [32] 27, wherein an intramolecular inversion of DNA sequences located between inverted repeated *six* sites is obtained.

37. (Third Amendment) A method according to claim [32] 27, wherein an intramolecular deletion of a DNA sequence located between two directly oriented *six* sites is obtained.

38. (Fourth Amendment) A method according to claim [32] 27, wherein an intramolecular inversion of a DNA sequence located between two inversely oriented *six* sites is obtained.

39. (Fourth Amendment) A method according to claim [32] 27, wherein an intramolecular deletion of a DNA sequence located between direct repeated DNA sequences containing *six* sites is obtained.

40. (Fourth Amendment) A method according to claim [32] 27, wherein an intramolecular inversion of a DNA sequence located between inverted repeated DNA sequences containing *six* sites is obtained.

41. (Twice Amended) A method according to claim 35, wherein the [specific recognition sequence is] DNA sequences are located within an extrachromosomal DNA substrate.

42. (Twice Amended) A method according to claim 36, wherein the [specific recognition sequence is] DNA sequences are located within an extrachromosomal DNA substrate.

43. (Fourth Amendment) A method for catalyzing site-specific resolution of DNA sequences located between *six* sites in an extrachromosomal substrate transfected into [a] an *in vitro* mammalian cell, comprising the step of catalyzing the site-specific resolution with prokaryotic beta recombinase derived from *Streptococcus*; wherein [the mammalian cell provides factors which beta recombinase is capable of using in order to mediate recombinase activity] recombination occurs between *six* sites.

50. (Third Amendment) A method according to claim [43] 66, wherein the *six* sites are wrapped on a nucleosome at several locations.

53. (Fourth Amendment) A method for mediating transgenic intramolecular recombination in *in vitro* mammalian cells, comprising the [step] steps of transfecting mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mammalian cells in order to mediate recombinase activity; and wherein the] recombination occurs between *six* sites and in the presence of cell factors [provided by the mammalian cells comprise] comprising HMG1 chromatin-associated protein.

55. (Fourth Amendment) A method for mediating transgenic intramolecular recombination in chromatin structures of mammalian cells, comprising the [step] steps of transfecting *in vitro* mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mammalian cells; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mammalian cells in order to mediate recombinase activity; and wherein the] recombination occurs between *six* sites and in the presence of cell factors [provided by the mammalian cells comprise] comprising HMG1 chromatin-associated protein.

56. (Twice Amended) A method according to claim 28, wherein an intramolecular deletion of DNA sequences located between direct repeated *six* sites [in the chromatin structures] is obtained.

57. (Twice Amended) A method according to claim 28, wherein an intramolecular inversion of DNA sequences located between inverted repeated *six* sites [in the chromatin structures] is obtained.

60. (Fourth Amendment) A method of mediating beta recombinase activity comprising the [step] steps of transfecting *in vitro* mammalian cells with prokaryotic beta recombinase derived from *Streptococcus* and transfecting the mammalian cells with DNA sequences containing *six* sites that allow recombination activity; wherein [the beta recombinase is capable of using mammalian cell factors of the mammalian cells to mediate recombinase activity] recombination occurs between *six* sites.

61. (Twice Amended) A method according to claim 60, wherein [the mammalian cell factors comprise] recombination occurs in the presence of cell factors comprising HMG1 chromatin-associated protein.

64. (Amended) A method for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in mouse cells, comprising the [step] steps of transfecting mouse cells with prokaryotic beta recombinase derived from Streptococcus and transfecting the mouse cells with DNA sequences containing *six* sites that allow recombination activity; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mouse cells in order to mediate recombinase activity] recombination occurs between two *six* sites.

65. (Amended) A method for mediating transgenic intramolecular recombination selected from deletions of DNA sequences located between two *six* sites and inversions of DNA sequences located between two *six* sites, in [chromatin structures of] mouse cells, comprising the [step] steps of transfecting mouse cells with prokaryotic beta recombinase derived from *Streptococcus* and integrating DNA sequences containing *six* sites that allow recombination activity into chromatin of the mouse cells; wherein [the prokaryotic beta recombinase is capable of using factors provided by the mouse cells in order to mediate recombinase activity] recombination occurs between two *six* sites.